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Mamiya et al., JOURNAL OF BIOLOGICAL CHEMISTRY (1999 May 28) 274 (22) 15751-6. Thanks!

Laurie Scheiner
Art Unit 1648
CM1 8E05
308-1122

THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 274, No. 22, Issue of May 28, pp. 15751–15756, 1999
Printed in U.S.A.

Hepatitis C Virus Core Protein Binds to a DEAD Box RNA Helicase*

(Received for publication, December 8, 1998, and in revised form, March 2, 1999)

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Approximately 4 million Americans are infected with the hepatitis C virus (HCV), making it a major cause of chronic liver disease. Because of the lack of an efficient cell culture system, little is known about the interaction between HCV and host cells. We performed a yeast two-hybrid screen of a human liver cell cDNA library with HCV core protein as bait and isolated the DEAD box protein DBX. DBX has significant amino acid sequence identity to mouse PL10, an ATP-dependent RNA helicase. The binding of DBX to HCV core protein occurred in an *in vitro* binding assay in the presence of 1 M NaCl or detergent. When expressed in mammalian cells, HCV core protein and DBX were co-localized at the endoplasmic reticulum. In a mutant strain of *Saccharomyces cerevisiae*, DBX complemented the function of Ded1p, an essential DEAD box RNA helicase. HCV core protein inhibited the growth of DBX-complemented mutant yeast but not Ded1p-expressing yeast. HCV core protein also inhibited the *in vitro* translation of capped but not uncapped RNA. These findings demonstrate an interaction between HCV core protein and a host cell protein involved in RNA translation and suggest a mechanism by which HCV may inhibit host cell mRNA translation.

Hepatitis C virus (HCV)[1] was discovered by cDNA cloning in 1989 and shown to cause chronic liver disease (1, 2). Approximately 4 million Americans and 150 million individuals worldwide are infected with HCV and at risk for cirrhosis and hepatocellular carcinoma (3–6). Because development of a robust cell culture system for HCV infection has remained elusive (6), extremely little is known about HCV-host cell interactions and how they influence cell physiology or viral replication.

HCV is a positive single-stranded RNA virus and a member of the *Flaviviridae* family (1, 7–10). Once HCV infects cells, the positive, single-stranded RNA genome is translated into a polyprotein of 3010 to 3033 amino acids, depending upon the strain (7–10). The viral RNA is not capped, and translation occurs via an internal ribosome entry site at the 5′ end of the viral RNA (11, 12). The mechanism of translation of uncapped viral RNA therefore differs from that used by virtually all cellular mRNAs that are capped at their 5′ ends. The HCV polyprotein is cleaved by both host cell and viral proteases into several smaller polypeptides (7–10, 13). The major structural proteins are a core protein and two envelope proteins called E1 and E2. The core protein forms the nucleocapsid of the mature virion, and E1 and E2 are present in the viral envelope. A small polypeptide called P7 is also generated as a result of cleavage at the E2-NS2 junction, but its function is not clear. Four major nonstructural proteins called NS2, NS3, NS4, and NS5 are also generated, two of which, NS4 and NS5, are further processed into smaller polypeptides called NS4A, NS4B, NS5A, and NS5B. Most of the nonstructural proteins have enzymatic activities that are critical for viral replication.

After cells are infected with a virus, viral proteins can interact with host cell proteins and influence cell physiology. In previous studies, HCV core protein has been shown to bind to lymphotoxin-β receptor and other tumor necrosis factor receptor family members (14, 15). A truncated form of HCV core protein also interacts with ribonucleoprotein K in the nucleus (16). We now show that HCV core protein binds to a cellular RNA helicase and, in experimental systems, inhibits capped RNA translation. This provides a novel mechanism by which HCV may inhibit mRNA translation in infected cells or recruit a cellular protein to enhance its own replication.

EXPERIMENTAL PROCEDURES

Yeast Two-Hybrid Screening—The Matchmaker Two-Hybrid System 2 was used to screen human liver Matchmaker cDNA library HL4002AB (CLONTECH) with the cytoplasmic domain (amino acids 1 to 123, which precede the first predicted transmembrane segment) of HCV core protein as bait in the yeast two-hybrid assay (17). Library screening was performed using previously described methods (18, 19). To construct the bait plasmid, DNA encoding amino acids 1–123 of HCV core protein (numbering as in Ref. 8) was amplified by PCR with pHCV-1 (13), provided by M. Houghton (Chiron Corp.) as template. The HCV sequences in pHCV-1 derive from a library made from the plasma of an infectious chimpanzee (13). The amplified DNA was cloned into the GAL4 DNA binding domain fusion vector pAS2–1 (CLONTECH) to yield pAS2–1-HCV-core$_{1-123}$. *Saccharomyces cerevisiae* strain Y190 was sequentially transformed with pAS2–1-HCV-core$_{1-123}$ and library recombinants in the GAL4 activation domain fusion vector pACT2 (CLONTECH). Positive pACT2-derived plasmids were rescued and used to co-transform yeast with pAS2–1-HCV-core$_{1-123}$, pLAM5′-1 (CLONTECH), and pAS2–1 to confirm the specificity of the reactions. For analysis of PL10 and Ded1p binding, cDNAs encoding PL10 from amino acids 408 to 660 and Ded1p amino acids 368 to 604 (corresponding to the longest portion of DBX isolated in the two-hybrid screen) were amplified by PCR from template plasmids (20), provided by T.-H. Chang (Ohio State University). The amplified cDNAs were cloned into pACT2 and used to co-transform yeast with pAS2–1-HCV-core$_{1-123}$. DNA sequencing of isolated library plasmid inserts and the bait constructs was performed on a 373A Sequencer (Applied Biosystems) at the Columbia University Cancer Center DNA core facility. Sequence analysis was performed using the Wisconsin Package (Genetics Computer Group)

* This work was supported by a grant from the Blowitz-Ridgeway Foundation. The confocal microscopy facility used for this work was established by National Institutes of Health (NIH) Grant 1S10 RR10506 and supported by NIH Grant 5 P30 CA13696 as part of the Herbert Irving Cancer Center at Columbia University. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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[1] The abbreviations used are: HCV, hepatitis C virus; GST, glutathione *S*-transferase; GPD, glyceraldehyde-3-phosphate; PCR, polymerase chain reaction.

and applications available via the internet at the National Center for Biotechnology Information World Wide Web site.[2]

In Vitro Binding Assays—A PCR product encoding the cytoplasmic domain of HCV core protein (amino acids 1–123) was cloned into pBFT4 for *in vitro* transcription-translation (21). *DBX* cDNA encoding amino acids 409 to 662 was excised from plasmid pACT2 by restriction endonuclease digestion and cloned into pGEX2T (Amersham Pharmacia Biotech) to yield pGEX2T-DBX$_{409-662}$, which expressed a glutathione *S*-transferase (GST) fusion protein in *Escherichia coli*. Plasmid construction was confirmed by DNA sequencing. *In vitro* transcription-translation was performed with the TNT T7 Coupled Reticulocyte Lysate System (Promega) using L-[^{35}S]methionine (NEN Life Science Products). Binding assays were performed as described previously (21).

Cell Transfection and Confocal Immunofluorescence Microscopy—A PCR product encoding full-length HCV core protein (amino acids 1–191) obtained using pHCV-1 (13) as template was cloned in-frame into pBFT4, which contains an initiation codon and FLAG tag 5′ to the cloning site. A DNA fragment was isolated by restriction endonuclease digestion at sites flanking the initiation and termination codons and cloned into pSVK3 (Amersham Pharmacia Biotech) to obtain pSVK3-FLAG-core for expression of HCV core protein with a FLAG tag at its amino terminus. To obtain full-length *DBX* cDNA, PCR was performed using a Marathon-ready cDNA human liver library (CLONTECH) as template to amplify the first 1439 nucleotides of *DBX* cDNA, which was ligated in-frame into pGEX2T-DBX$_{409-662}$ to produce pGEX2T-DBX. The coding region of pGEX2T-DBX was isolated by restriction endonuclease digestion and cloned into pBluescript II SK$^-$ (Stratagene) to produce pBluescript-DBX. A cDNA containing the 3′ 668 nucleotides of DBX, excluding the stop codon, was amplified by PCR and ligated into pBluescript II-DBX to replace the corresponding nucleotides. The entire DBX coding region was then excised by restriction endonuclease digestion and ligated into pcDNA3.1 (-)/Myc-His A (Invitrogen) to produce pcDNA3.1/His A-DBX-myc, that encoded full-length DBX with a c-myc tag at its carboxyl terminus. All plasmid constructs were confirmed by DNA sequencing. HeLa or COS-7 cells (ATCC) grown on glass slides were transfected with pSVK3-FLAG-core, pcDNA3.1/HIS A-DBX-myc or both using Tfx-20 (Promega), or DMRIE-C (Life Technologies, Inc.). Cells were washed in phosphate-buffered saline 48 h after transfection and fixed with methanol for 5 min at −20 °C followed by acetone at −20 °C for 20 s. Indirect immunofluorescence microscopy was performed as described (22). To detect express FLAG-tagged proteins in double-labeling experiments, FLAG-probe (Santa Cruz Biotechnology), a rabbit polyclonal antibody, was used. To reduce background, FLAG-probe was incubated with COS-7 cells fixed with methanol/acetone at a 1:100 dilution for 12–16 h before use. Anti-FLAG M2 monoclonal antibody (Eastman Kodak Co.) was used in single-labeling experiments at a 1:200 dilution. Monoclonal anti-c-myc antibody 9E10 (Babco) was used at a 1:1000 dilution. Fluorescein isothiocyanate-conjugated goat anti-rabbit IgG and rhodamine-conjugated goat anti-mouse IgG secondary antibodies were obtained from Jackson Immuno Research Laboratories. Microscopy was performed using a Zeiss LSM 410 confocal laser scanning system attached to Zeiss Axiovert 100TV inverted microscope (Carl Zeiss). Images were processed using Photoshop software (Adobe) on a Macintosh G3 computer (Apple Computer).

Yeast Strains—Yeast strain YTC83 [MATa *ded1::TRP1 ura3–52 lys2–801 ade2–101 trp1-Δ1 his3-Δ200 leu2-Δ1* pPL1004 *(PL10/CEN/LEU2)*], which contains a chromosomal *ded1* deletion complemented by *PL10* cDNA (20), was provided by T.-H. Chang. To obtain a yeast strain with a chromosomal *ded1* deletion complemented by *DBX* cDNA, full-length *DBX* cDNA was excised from pGEX2T-DBX by restriction endonuclease digestion and ligated into pRS315pG1 (provided by T.-H. Chang). This plasmid was used to transform yeast strain YTC75 [MATa *ded1::TRP1 ura3–52 lys2–801 ade2–101 trp1-Δ1 his3-Δ200 leu2-Δ1* pDED1008 *(DED1/CEN/URA3)*] (20), provided by T.-H. Chang, which was then grown on leucine dropout plates. Transformants were replica-plated onto 5-fluoroorotic acid plates as described (23) to yield strain YNM1DX. To obtain a yeast strain with a chromosomal *ded1* deletion complemented by *DED1* cDNA driven by a glyceraldehyde-3-phosphate (GPD) promoter on a centromeric plasmid, the native promoter, 5′-untranslated region, and part of the 5′-coding region were excised by restriction endonuclease digestion from pDED1009 (*DED1/CEN/LEU2*) (provided by T.-H. Chang). The GPD promoter, isolated from pRS315pG1 by restriction endonuclease digestion, and 477 5′-coding nucleotides of *DED1*, amplified by PCR, were then sequentially ligated into this pDED1009-derived plasmid to yield pDED$_{GPD}$. Yeast strain YTC75 was then transformed with pDED$_{GPD}$, and 5-fluoroorotic acid counter-selection was performed to obtain strain YNM1DD [MAT a *ded1::TRP1 ura3–52 lys2–801 ade2–101 trp1-Δ1 his3-Δ200 leu2-Δ1* pDED$_{GPD}$ *(DED1/CEN/LEU2)*]. All constructs were confirmed by DNA sequencing.

Effects of HCV Core Protein on Growth of Yeast Strains—The coding region for full-length HCV core protein (amino acid 1–191) was excised from pBFT4 by restriction endonuclease digestion and ligated into p423GPD (ATCC) to produce p423GPD-core. The coding region for the cytoplasmic domain of HCV core protein (amino acid 1–123) was also ligated into p423GPD to yield p423GPD-core$_{1-123}$. Constructs were confirmed by DNA sequencing. Yeast strains YTC83, YNM1DX, and YNM1DD were transformed with p423GPD, p423GPD-core, and p423GPD-core$_{1-123}$ using the lithium acetate-mediated method (24) and grown on histidine-leucine dropout plates for 7 days. Plates were photographed to record colony growth.

Effect of HCV Core Protein on in Vitro Translation—cDNA encoding HCV core protein from amino acids 1 to 123 was ligated into pGEX4T-3 (Amersham Pharmacia Biotech) to produce a GST fusion protein (GST-core$_{1-123}$) in *E. coli*. Plasmid construction was confirmed by DNA sequencing. pGEM-luc (Promega) was linearlized with *Xho*I and used as a template for luciferase RNA transcription with the RiboMAX RNA Production System-SP6 (Promega). When capped RNA was synthesized, 3 mM ^{7}mGpppG (New England Biolabs) was included in the reaction mixture. The DNA template was removed by digestion with DNase following the transcription reaction, and synthesized mRNA was purified using the RNeasy Mini Kit (Qiagen). For *in vitro* translation, 16.5 μl of Flexi Rabbit Reticulocyte Lysate (Promega) was used and incubated for 1 h at 4 °C with 8.25 μl of glutathione-Sepharose 4B (Amersham Pharmacia Biotech) loaded with either 300 ng of GST-core$_{1-123}$ or GST followed by centrifugation for 5 min at 2000 × *g*. Translation reactions were then performed according to the manufacturer's instructions, and luciferase activity was measured by luminescence emission using the Luciferase Assay System (Promega).

RESULTS

HCV Core Protein Binding to DBX—Screening of 8 × 10^6 recombinants of a human liver cell cDNA library with the cytoplasmic domain of HCV core protein as bait in the yeast two-hybrid assay led to the isolation of 5 positive clones, 3 of which encoded portions of DBX, the longest from amino acid 409 to amino acid 662. The 2 other positive clones encoded portions of epsilon 14-3-3, a member of the 14-3-3 family of proteins that has numerous proposed functions, including activities in signal transduction. DBX is the human orthologue of the mouse DEAD box protein PL10 (25–27). PL10 is the functional orthologue of *S. cerevisiae* Ded1p, an ATP-dependent RNA helicase for capped mRNA (20). DBX is 95% identical in primary structure to PL10 and 54% identical to Ded1p (Fig. 1*A*). In the yeast two-hybrid assay, HCV core protein interacts with DBX and PL10 but not with Ded1p (Fig. 1*B*).

We confirmed the interaction between HCV core protein and DBX in an *in vitro* binding assay. The cytoplasmic domain of HCV core protein was synthesized by *in vitro* translation and incubated with GST or a GST fusion protein containing DBX from amino acid 409 to amino acid 662. Proteins were precipitated with glutathione-Sepharose, and HCV core protein binding was analyzed by autoradiography. HCV core protein did not bind to GST but did bind to GST-DBX fusion protein in buffers containing NaCl concentrations as high as 1 M (Fig. 2*A*). Binding also occurred in buffers containing 1% of the nonionic detergent Nonidet P-40 (Fig. 2*B*).

Co-localization of HCV Core Protein and DBX in Cells—An interaction between HCV core protein and DBX in mammalian cells was further supported by their intracellular co-localization. Indirect confocal immunofluorescence microscopy of transfected HeLa cells showed that full-length HCV core protein, which contains the cytoplasmic domain and a single transmembrane segment, was localized to the endoplasmic reticulum in discrete foci (Fig. 3*A*). A similar localization in the endoplasmic reticulum has been reported by others (13). Focal aggregates of HCV core protein likely arise because this

[2] http://www.ncbi.nlm.nih.gov/.

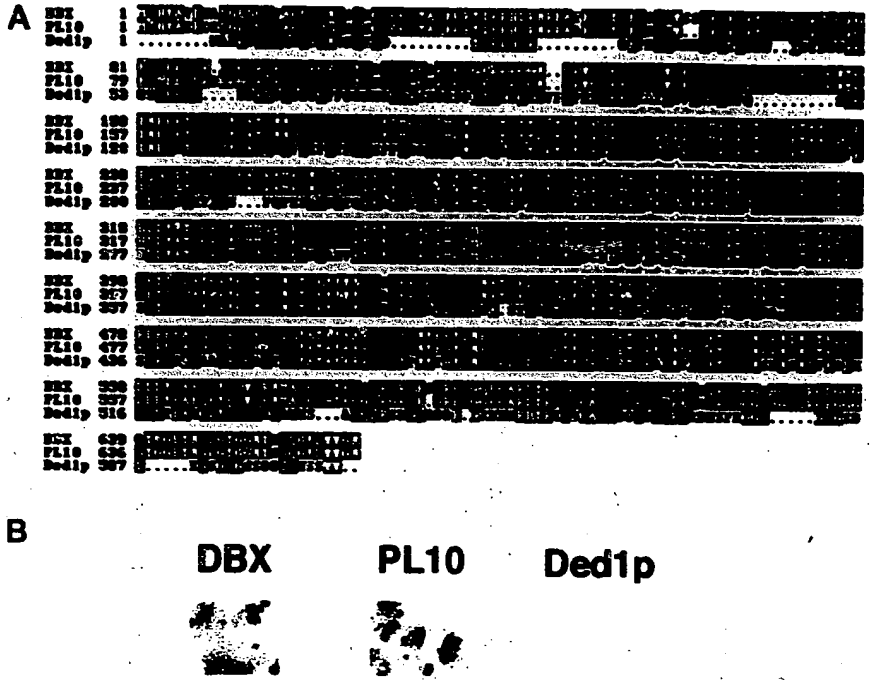

FIG. 1. **Primary structures of DBX, PL10, and Ded1 and their interactions with HCV core protein in the yeast two-hybrid assay.** *A*, alignment of deduced amino acid sequences of DBX (GenBank® accession number AF000982), PL10 (GenBank® accession number J04847), and Ded1p (GenBank® accession number X57278) is shown. Identical amino acids are shown as *white* on *cyan*. Conserved substitutions are shown as *black* on *magenta*. *Dots* represent gaps to optimize alignments, which were obtained using the Pileup program. *B*, two-hybrid assays showing interaction of HCV core protein with DBX and PL10 but not with Ded1p. Yeast strain Y190 was co-transformed with a plasmid expressing the cytoplasmic domain of HCV fused to the GAL4 DNA binding domain and plasmids expressing either a portion of DBX or the corresponding portions of PL10 or Ded1p fused to the GAL4 transcriptional activation domain. Transformants giving β-galactosidase activity (positive interactions) are *blue*. Control reactions of DBX, PL10, and Dep1p GAL 4 activation domain fusion proteins with GAL4 DNA binding domain alone were negative (data not shown).

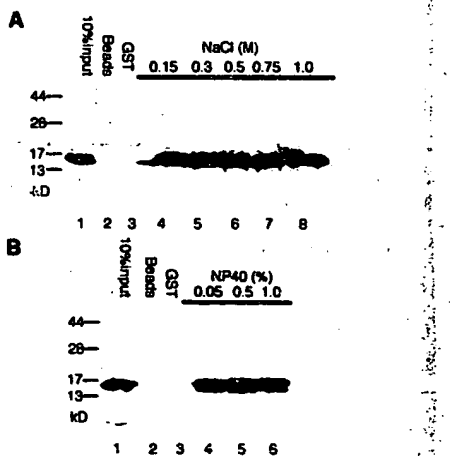

FIG. 2. **Binding of DBX to HCV core protein *in vitro*.** *A*, a standard amount of ^{35}S-HCV core protein (amino acids 1–123), 10% of which is shown in the autoradiogram (*lane 1*), was used in each binding assay. ^{35}S-HCV core protein was incubated with glutathione-Sepharose (*lane 2*): 20 μg of GST coupled to glutathione-Sepharose (*lane 3*) in binding buffer containing 0.15 M NaCl and 0.2 μg of GST-DBX fusion protein coupled to glutathione-Sepharose in buffers containing the NaCl concentrations indicated above each *lane* (*lanes 4–8*). Glutathione-Sepharose was then washed with buffer containing the indicated NaCl concentration, and the bound proteins were eluted with 4% SDS, subjected to SDS-polyacrylamide gel electrophoresis, and detected by autoradiography of dried slabs gels. *B*, binding assay similar to that shown in *panel A* in which GST-DBX fusion protein was incubated with ^{35}S-HCV core protein in buffers containing 0.15 M NaCl and 0.05 to 1.0% of Nonidet P-40 (*lanes 4–6*). Migrations of molecular mass standards are indicated in kilodaltons at the left of each *panel*.

polypeptide multimerizes (28). In cells not expressing HCV core protein, DBX had a more diffuse cytoplasmic distribution (Fig. 3*A*). In cells expressing HCV core protein, however, DBX was found in most instances in discrete foci that co-localized with HCV core protein (Fig. 3*B*). The antibodies used to detect the respective epitope tags of each protein did not cross-react significantly (Fig. 3*B*). HCV core protein therefore forms aggregates at the endoplasmic reticulum membrane with which DBX apparently associates.

DBX Rescues Ded1-deletion Yeast Mutants and Rescue Is Prevented by HCV Core—DBX likely functions as an ATP-dependent RNA helicase for cellular mRNA, which can be inferred from its sequence similarity to mouse PL10 and yeast Ded1p (20, 27). To examine the effect of HCV core protein on DBX function, we took advantage of yeast genetics and the fact that *S. cerevisiae* has only one essential DBX-like protein, Ded1p (20). When driven by a yeast GPD promoter and carried on a centromere plasmid, mouse *PL10* cDNA, as described previously (20), and *DBX* cDNA rescued the lethality of cells with a chromosomal *ded1* deletion. This indicates that DBX can likely function as a RNA helicase as it can replace the function of the yeast DEAD box RNA helicase Ded1p. Expression of full-length HCV core protein severely inhibited the growth of *DBX*- and *PL10*- complemented *ded1*-deletion yeast but not *ded1*-deletion yeast complemented with *DED1* cDNA driven by the same promoter on a centromeric plasmid (Fig. 4). This is consistent with the observation that DBX and PL10, but not Ded1p, bind to HCV core protein. The cytoplasmic domain of HCV core protein that binds to DBX, without a transmembrane segment, did not significantly inhibit the growth of *DBX*- and *PL10*- complemented *ded1*-deletion yeast (data not shown), suggesting that inhibition of function may result from trapping of these proteins in aggregates at the endoplasmic reticulum membrane (see Fig. 3).

Inhibition of in Vitro Translation of Capped mRNA by HCV Core Protein—We examined the effects of HCV core protein on the translation of capped and uncapped luciferase RNA in an *in vitro* reticulocyte lysate assay. If HCV inhibits the function of DBX as a RNA helicase, it should theoretically decrease the translation of capped RNA but not significantly affect the translation of uncapped RNA. In the *in vitro* translation assay, the cytoplasmic portion of HCV core protein significantly inhibited the *in vitro* translation of luciferase from capped but not uncapped RNA (Fig. 5). Capped RNA translation was approximately 4-fold higher than uncapped RNA translation in this assay (data not shown). This finding suggests that HCV

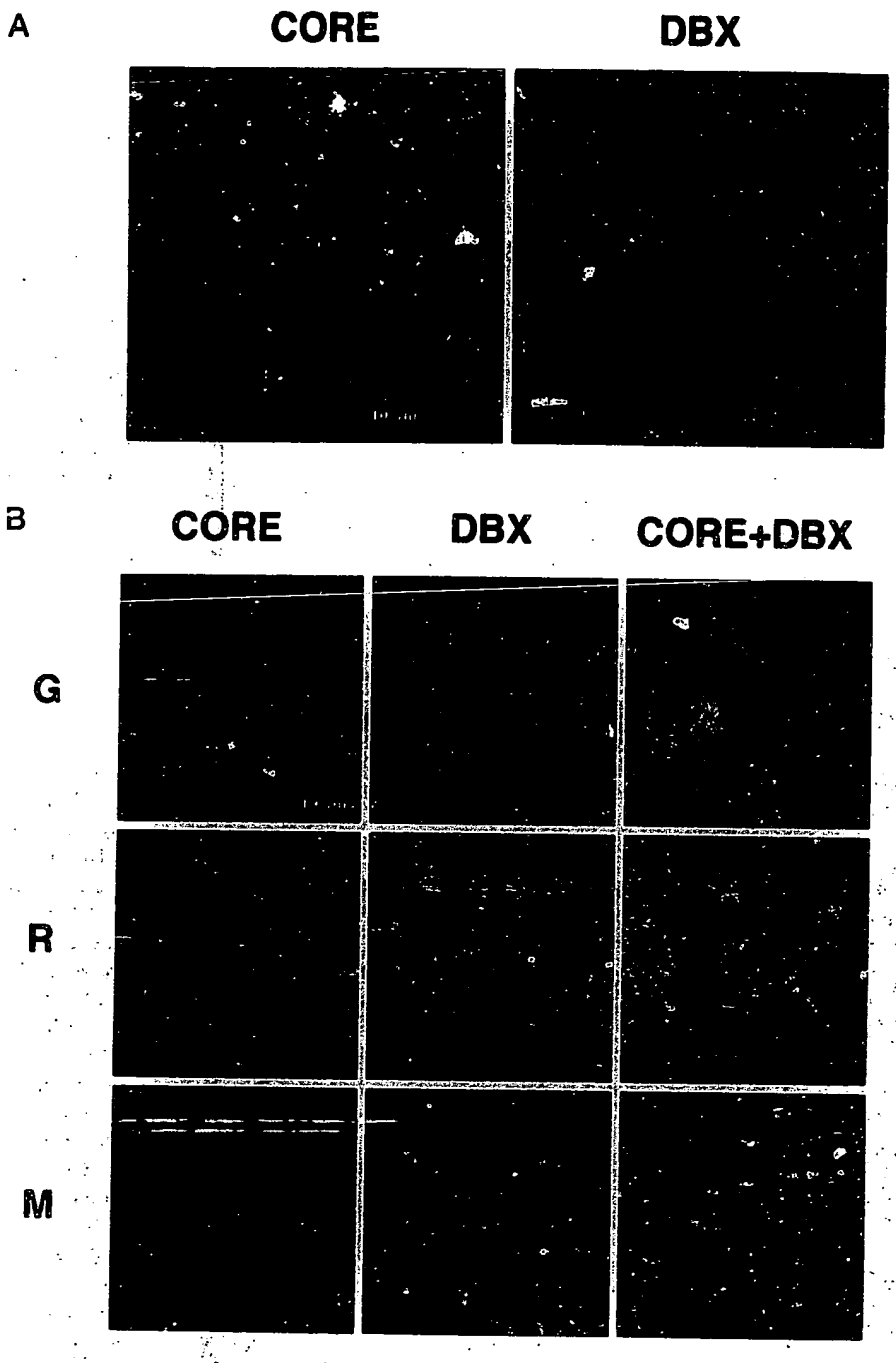

FIG. 3. **Immunofluorescence localization of DBX and HCV core protein in mammalian cells.** *A*, HeLa cells were transiently transfected with cDNA encoding FLAG-tagged HCV core protein (*left panel*) or with cDNA encoding myc-tagged DBX (*right panel*). Cells were incubated with monoclonal anti-FLAG or anti-myc (9E10) antibody followed by rhodamine-conjugated secondary antibody. HCV core protein appears primarily in large, discrete foci at the endoplasmic reticulum membrane, whereas DBX has a more diffuse cytoplasmic localization. *B*, co-localization of DBX and HCV core protein in COS-7 cells transiently transfected to express both FLAG-tagged HCV core protein and myc-tagged DBX. All cells were fixed and incubated with the same combination of rabbit anti-FLAG polyclonal antibody and mouse anti-myc monoclonal (9E10) antibody followed by both fluorescein isothiocyanate-conjugated goat anti-rabbit and rhodamine-conjugated goat anti-mouse antibodies. Cells transfected to express FLAG-tagged HCV core protein alone (*left column*) showed essentially only *green* fluorescence, resulting from anti-FLAG and fluorescein isothiocyanate-conjugated antibody labeling (*row G*). Cells transfected to express myc-tagged DBX alone (*middle column*) showed essentially only *red* fluorescence, resulting from anti-myc and rhodamine-conjugated antibody labeling (*row R*). The *right column* shows COS-7 cells co-transfected to express both FLAG-tagged HCV core protein (*row G*) and myc-tagged DBX (*row R*). Merged images (*row M*) appear *yellow* where *green* fluorescence corresponding to HCV core protein localization and *red* fluorescence corresponding to DBX localization overlap.

core protein may inhibit the translation of capped mRNA in cells, presumably by inhibiting DBX function.

DISCUSSION

HCV core protein binds to the human DEAD box protein DBX. DBX rescues the lethal phenotype of *ded1*-deletion, demonstrating that it can function as a RNA helicase for capped mRNA, replacing the essential yeast DEAD box RNA helicase Ded1p. Our findings that HCV core protein prevents DBX from rescuing *ded1*-deletion yeast and that it inhibits the translation of capped RNA *in vitro* strongly suggest that it may inhibit cellular mRNA translation *in vivo*. These results, however, cannot establish if translation inhibition occurs as a result of HCV core protein inhibiting DBX RNA helicase activity *per se* or by an interaction that results in "trapping" DBX at a location near the membrane of the endoplasmic reticulum where in cannot function properly. Inhibition of host cell mRNA translation could theoretically provide viral RNA molecules with enhanced access to ribosomes and the rest of the protein synthesis machinery of the cell, a phenomenon shared by several different viruses (29). A recent report has shown that high levels of expression of HCV structural and nonstructural proteins is toxic to mammalian cells (30); however, it is not clear if this toxicity results from inhibition of host cell translation. Because the development of a robust cell culture system to study HCV has remained elusive, it would be extremely difficult to directly investigate the effects of HCV infection on host cell mRNA translation. Despite these methodological constraints limiting the ability to directly test the hypothesis, our discovery that HCV core binds to DBX and inhibits capped RNA translation in experimental assays suggests that it can similarly inhibit mRNA translation in infected human cells.

DEAD box RNA helicases unwind capped mRNA (20), and

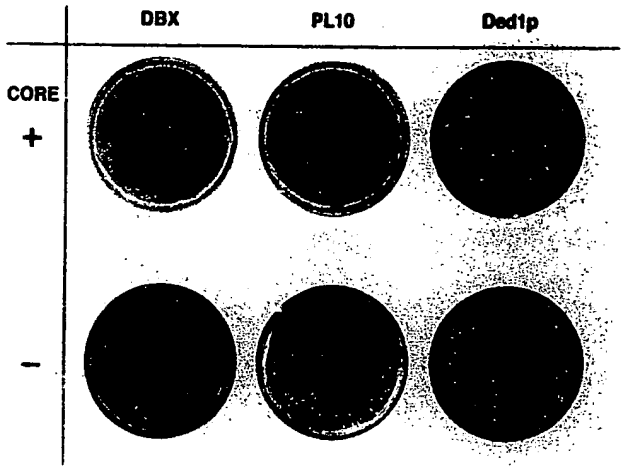

FIG. 4. **Inhibition of DBX and PL10 but not Ded1p by HCV core protein.** Yeast strains with chromosomal *ded1* deletion complemented with either *DBX*, *PL10*, or *DED1* cDNAs driven by the yeast GPD promoter on centromeric plasmids were transformed with a plasmid that expressed full-length HCV core protein (*top*) or control plasmid p423GPD (*bottom*). The resulting transformants were spread on histidine, leucine drop-out plates and incubated at 30 °C for 7 days, and photographs (negatives are shown) were taken of each plate. Note colony growth of all yeast strains transfected with control plasmid (*bottom panels*). In contrast, *DBX*- and *PL10*- complemented *ded1*-deletion strains do not demonstrate significant colony growth when HCV core protein is expressed, whereas growth of the *DED1*-complemented strain is unaffected (*top panels*).

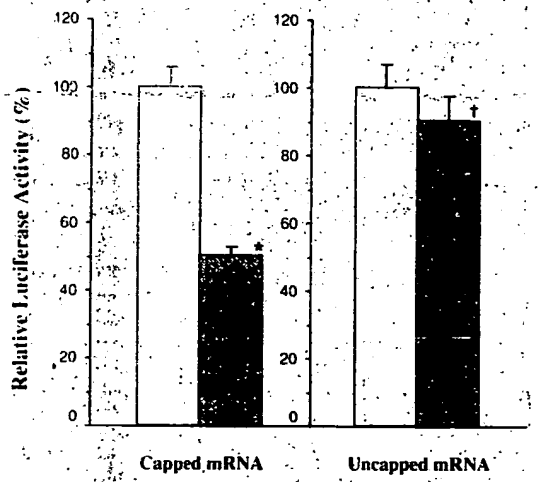

FIG. 5. **Inhibition of translation of capped mRNA *in vitro* by HCV core protein.** Rabbit reticulocyte lysates were incubated with glutathione-Sepharose beads loaded with either 300 ng of a GST-HCV core fusion protein or GST. *In vitro* synthesized capped or noncapped luciferase mRNAs were translated at 30 °C for 90 min, and luciferase activity was measured. Results are expressed as the relative luciferase activities produced in reticulocyte lysate-treated equal concentrations of GST-HCV core fusion protein (*shaded bars*) or GST (*open bars*, arbitrarily assigned 100% activity). Values shown are means ±S.E. ($n = 6$). *$p < 0.0001$; †, no significant difference.

inhibition of their function should decrease translation of cellular mRNA. Inhibition of DBX function by HCV core protein may only partially inhibit host mRNA translation in mammalian cells because they contain other putative RNA helicases (31). In contrast, the translation of HCV RNA, which is not capped, utilizes internal ribosome entry sites (11, 12), and can be unwound by its own RNA helicase, which is part of the HCV NS3 protein (32, 33), and may proceed without DBX. This hypothetical mechanism is reminiscent of that used by poliovirus, which inhibits translation factor eIF-4F (34, 35) and also has RNA with internal ribosome entry sites (36). In cells, eIF-4F exists as a complex with eIF-4B, which has RNA binding activity, and eIF-4A, which is also a DEAD box RNA helicase (37). HCV and poliovirus infection may both therefore cause a decrease in the unwinding of capped mRNA in host cells.

In addition to inhibiting capped mRNA translation in infected host cells, the interaction between HCV core protein and DBX may play other possible roles, including the recruitment of DBX to participate in HCV replication itself. Recruitment of host cells proteins into virions to enhance viral replication has been demonstrated in other systems. For example, the principal structural protein of the human immunodeficiency virus HIV-1 binds to cyclophilins and recruits cyclophilin A into viral particles, which appears to be necessary for efficient viral replication (38, 39). In a similar fashion, recruitment of DBX into HCV particles by binding to core protein may enhance viral replication. This could theoretically occur by DBX altering viral genomic RNA structure in viral particles in newly infected cells. Testing of this hypothesis is limited at the present time because of the lack of an efficient cell culture system for HCV.

HCV core protein has also been shown to bind to lymphotoxin-β receptor and other tumor necrosis factor receptor family members (14, 15) as well as ribonucleoprotein K (16). In our yeast two-hybrid screen, we did not isolate clones for these proteins, possibly because of subtle differences in our bait construct and the different cDNA library we used. The demonstration that other proteins interact with HCV core protein suggests that its expression in cells may have myriad consequences. Other groups (40, 41) have also reported that HCV core protein represses transcription from the p53 promoter and other eukaryotic promoters. The overall effect of HCV core protein on cell physiology under natural conditions of infection is, however, difficult to assess at the present time because of lack of a cell culture system for HCV.

Finally, it should be noted that the best current treatment regimens for chronic hepatitis C are effective in only a minority of patients (42). If interactions between HCV and host cell proteins alter cell survival or enhance viral replication, they could be rational targets for antiviral drug design. Regardless of the physiological significance, the tight binding of any polypeptide to a structural or nonstructural protein of HCV may potentially interfere with viral replication. The identification of polypeptides such as DBX that bind to HCV proteins therefore has implications for the design of compounds which may be therapeutically useful in the treatment of patients with chronic hepatitis C.

Acknowledgments—We thank T.-H. Chang (Ohio State University) for providing reagents and invaluable advice, S. P. Goff (Columbia University) for helpful discussions, M. Houghton (Chiron Corp.) for providing pHCV-1, and P. J. Mustacchia (Columbia University) and T. W. Chun (State University of New York) for reviewing the manuscript.

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